ELSEVIER

Cancer Letters 143 (1999) 189–194

CANCER LETTERS

Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer

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Received 3 November 1998; received in revised form 16 February 1999; accepted 17 February 1999

Abstract

High-temperature cooking techniques and doneness level of red meat are linked to cancer of various sites, particularly colorectal cancer. In a colorectal adenoma study, we found an elevated risk for red meat consumption that was mainly due to an association with well-done/very well-done red meat. High-temperature cooking methods (i.e. grilling) were also associated with increased risk. We are currently using an HCA database linked to this questionnaire to estimate MeIQx, DiMeIQx and PhIP consumption and determine their association with risk of colorectal adenoma. Similar results on red meat doneness and fried meat were found in a case-control study of lung cancer. Thus, initial positive findings are stimulating the development of a more refined questionnaire instrument and its validation using food diaries, 24-h recalls, biomarkers of internal dose and direct food measurements. Furthermore, the use of these exposure assessment approaches are being used in large prospective studies world wide and should help clarify the role of doneness, cooking practices and pyrolysis products in the etiology of human cancer.

Keywords: Heterocyclic amine; Epidemiology; Red meat; Cooking; Doneness; Questionnaire

Humans are exposed to heterocyclic amines when they consume diets containing meats cooked at high temperature. HCAs are formed when creatine or creatinine and amino acids in meat juice pyrolyze. Although HCAs are known mutagens and animal carcinogens, their carcinogenic potential in humans has not been established. To date, there are too few data to evaluate the strength, consistency, and dose response of the relationship between HCAs consumption and humans cancer [1] while there is ample evidence from in vitro and animal studies that HCAs can damage DNA and cause tumors of various organs in animal models [2–5]. To investigate cancer risk posed by HCAs to humans, accurate estimation of exposure is needed. We have developed methods to estimate how much HCAs people are consuming. The main route of human exposure to HCAs is through meat consumed in the diet. Thus, to estimate HCA exposure, we obtained information on usual level of meat consumption by methodologies similar to those used to assess other components of the diet. The primary method of long-term nutritional exposure assessment in epidemiological studies of chronic disease is through the use of food frequency questionnaires (FFQ) to estimate 'usual intake' of foods. To capture the total amount of HCAs consumed by an individual, we have developed meat cooking practice module within a FFQ, the Health Habits and History Questionnaire. This module obtains information from the subjects on usual intake of types of meat with the

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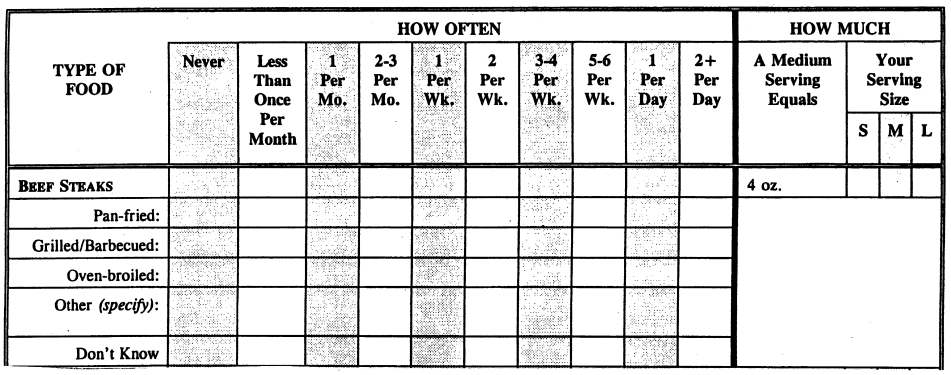
0304-3835/99/$ - see front matter
PII: S0304-3835(99)00123-8



TYPE OF FOOD	HOW OFTEN										HOW MUCH			
	Never	Less Than Once Per Month	1 Per Mo.	2-3 Per Mo.	1 Per Wk.	2 Per Wk.	3-4 Per Wk.	5-6 Per Wk.	1 Per Day	2+ Per Day	A Medium Serving Equals	Your Serving Size		
												S	M	L
BEEF STEAKS											4 oz.			
Pan-fried:														
Grilled/Barbecued:														
Oven-broiled:														
Other *(specify)*:														
Don't Know														

Fig. 1. An example of the meat-cooking module developed for use in epidemiologic studies.

portion size, method of cooking, degree of internal doneness and external browning (Fig. 1). Embedded within that are the different ways the steak is cooked. Furthermore, we obtained information on level of doneness both verbally (rare, medium-rare, medium, medium-well, well, and very well-done) and with photographs that showed internal doneness and external browning.

To estimate exposure, we need the HCA content of the various meat items. Therefore, we measured values of HCAs in commonly consumed meats subjected to representative cooking practices. Different types of meats were cooked by different methods to varying degrees of doneness. For example, beefsteak samples were cooked three different ways (pan-fried, oven-broiled or grilled/barbecued) at four degrees of doneness (rare, medium, well-done or very well-done). The other meats samples cooked similarly were hamburger patties, roast beef, bacon, sausage links, sausage patties, brown-and-serve sausage, ham steaks, pork chops, chicken breasts and thigh with and without skin and bones, roast chicken, and fish. Hamburger patties, breakfast sausage, fried chicken from fast-food restaurant chains and steaks, hamburger patties, and ribs from restaurants were also sampled [6–10]. Multiple samples were cooked for each specific meat type, method of cooking, and degree of doneness level, while for restaurants and fast-food chains multiple samples were obtained from different restaurants. All samples for one particular cooking method and doneness level were finely ground to form a composite sample. Levels of 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) were measured in each of the extracts from composite

Table 1
Top 10 MeIQx-containing meats in the population of the colon adenoma study

Meat type	Cooking method	Doneness level	% of MeIQx	MeIQx ng/100g of cooked meat
Gravy	Baked	Well-done	11	242
Hamburger patty	Pan-fried	Well-done	10	235
Hamburger patty	Pan-fried	Medium	9	100
Sausage patty	Pan-fried	Well-done	7	335
Hamburger patty	Grilled	Well-done	7	131
Steak	Grilled	Medium	7	64
Bacon	Pan-fried	Well-done	6	171
Chicken	Baked	Well-done	4	8
Pork chop	Pan-fried	Well-done	4	134
Steak	Pan-fried	Well-done	3%	407

Table 2
Top 10 PhIP-containing meats in the population of the colon adenoma study

Meat type	Cooking method	Doneness level	% of PhIP	PhIP ng/g of meat
Steak	Grilled	Medium	11	471
Skinless chicken	Grilled	Well-done	11	854
Skinless chicken	Grilled	Very well-done	8	7967
Steak	Broiled	Rare	7	614
Steak	Grilled	Rare	7	253
Steak	Grilled	Medium	7	471
Skinless chicken	Broiled	Very well-done	4	1478
Steak	Broiled	Medium	3	208
Chicken with skin	Broiled	Well-done	2	806
Skinless chicken	Broiled	Well-done	2%	196

samples by HPLC by the method of Knize et al. [8]. Using the information from the FFQ and the HCA database, we can estimate the amount of MeIQx and PhIP consumed in a given population. The top 10 meat items contributing to the consumption of MeIQx and PhIP in a colorectal adenoma study (described below) are presented in Tables 1 and 2.

As diet is a complex mixture containing carcinogens, co-carcinogens and anti-carcinogens, a reductionist approach of evaluating only HCAs may not be appropriate. We see many more peaks in the HPLC chromatogram for well-done meat than can be explained by MeIQx, DiMeIQx and PhIP (Fig. 2). Cooked meats may also contain other mutagens/carcinogens as well as saturated fats; a group of compounds which themselves have been implicated in carcinogenesis. The data from the FFQ responses should be analyzed by different methods, such as by meat group and by cooking practice category, as well as by level of estimated HCA intake, in order to determine which approach is appropriate for investigating the association with disease risk, and whether or not a particular approach produces consistent results across comparable studies.

A large number of epidemiological studies have evaluated the relationship between meat intake and risk of cancer. There is considerable heterogeneity in the characterization of meat intake across studies, however most of the evidence for an association with cancer risk comes from studies that considered red meat intake defined as beef, pork and lamb. A panel of experts from the World Cancer Research Fund (WCRF) and the American Institute recently reviewed studies of red meat intake and cancer risk. This panel of experts concluded that high intake of red meat *probably* increases the risk of developing colorectal cancer, and *possibly* increases the risk of pancreas, breast, prostate and kidney cancer [11].

Only a limited number of studies have evaluated the effects of cooking methods. The interpretation of results from theses studies is difficult due to the use of different terminology for cooking methods and the consideration of confounding factors such as meat itself, animal fat and animal protein. The WCRF panel of experts evaluated the epidemiologic evidence from these studies and concluded that: 'there is no convincing evidence that any method of cooking modifies the risk of any cancer, nor there is evidence of any probably causal relationship' [11]. However, they indicated that high intake of grilled or barbecued meat *possibly* increases the risk of stomach and colorectal cancer, and that there is *insufficient* evidence from a few studies that consumption of fried foods increases the risk of bladder cancer. A few studies have evaluated the role of meat cooking practices and provided some evidence for an association with an increased risk of various cancers [12–20]. However, data from these studies is sparse and no final conclusions can be drawn. A number of case-control and cohort studies that have incorporated new methodologies to estimate levels of HCA intake are currently being conducted and will be publishing results in the near future.

The food frequency questionnaire used in these studies was a modified version of the 100-item Health Habits and History Questionnaire, which obtained information on usual diet (frequency of consumption and portion size). In addition, we developed a meat-

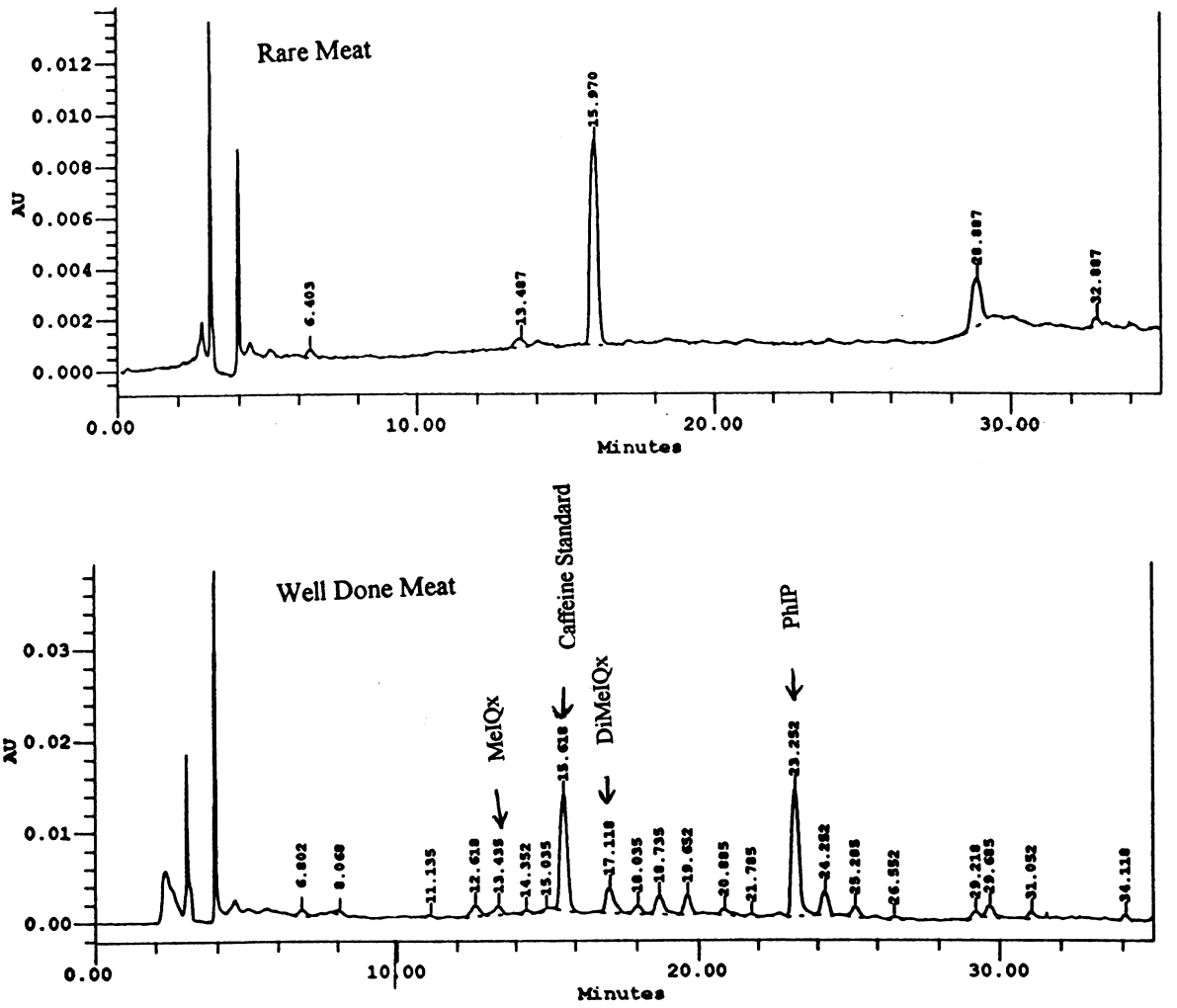


Fig. 2. Possible mutagenic compounds found in rare and well-done steak.

cooking module, which included 23 meat items. For meats prepared with variable cooking techniques we obtained information on the typical level of doneness and cooking method. Odds Ratios (ORs) and 95% confidence intervals were calculated using unconditional logistic regression. ORs were adjusted for other risk factor variables such as age, gender, total caloric intake, physical activity level, pack-years of cigarette smoking, use of non-steroidal anti-inflammatory drugs, etc.

Using the new questionnaire we have conducted three studies: colorectal adenoma, lung cancer, and breast cancer study. Colorectal cancer risk has been associated with increased consumption of red meat, and possibly with meat cooking practices. HCAs are formed in meats cooked at high temperatures, while polycyclic hydrocarbons and HCAs are mainly formed in grilled foods. To separate the roles of red meat from the pyrolysis products and the impact of susceptibility factors, we conducted a case-control study of colorectal adenomas on 146 newly diagnosed cases and 229 controls [15]. Meat and HCA intake were estimated from questions on meat consumption, cooking methods, doneness levels, and HCA database. An increase in consumption of red meat was associated with an elevation in risk. The increased

risk was mainly associated with well-done/very well-done red meat. High-temperature cooking methods were also associated with increase in risk. This suggests that some of the increased risk associated with red meat may be due to cooking practices.

Some epidemiological studies suggest that diets high in fat, saturated fat, or cholesterol are associated with increased risk of lung cancer. Since meat consumption is correlated with the intake of saturated fat and cholesterol, we investigated the role of meat intake and cooking practices in relation to lung cancer risk. We conducted a population-based case-control study of both non-smoking and smoking women in Missouri [16]. A 100-item food frequency questionnaire with detailed questions on meat consumption was completed by 593 cases and 623 frequency matched controls. We estimated quantity of meat eaten (grams/day) according to cooking method and doneness level. ORs and 95% confidence intervals (CIs) were calculated using logistic regression. Multivariate models included age, packyears of smoking, body mass index (BMI, kg/m^2), education, and intake of calories, fat, fruit/fruit juices, and vegetables. When comparing 90th and 10th percentiles, lung cancer risk increased for total meat consumption (OR = 1.6, CI 1.1–2.4), red meat (OR = 1.8, CI, 1.2-2.7), well-done red meat (OR = 1.5, CI, 1.1–2.1) and fried red meat (OR = 1.5, CI, 1.1–2.0). The odds ratios for 5th vs. 1st quintiles using the categorical variable for well-done red meat and fried red meat were essentially the same as reported above, however, the increase in risk was associated mainly with the 5th quintile. The ORs for a 10-g increase in consumption were 1.04 for total meat, 1.06 for red meat, 1.08 for well-done red meat, and 1.09 for fried red meat. Consumption of red meat, especially fried and/or well-done red meat, was associated with increased risk of lung cancer.

HCAs have also been demonstrated as mammary carcinogens in animals [15]. Furthermore, a nested case-control study among cohort members of the Iowa Women's Health was conducted by Zheng et al. [20]. A questionnaire with color photographs was mailed to breast cancer cases diagnosed from 1992 through 1994 and to a random sample of cancer-free cohort members to obtain information on usual intake of meats and meat preparation practices. Multivariate analysis was performed on 273 case subjects and 657 and a statistically significant dose–response relationship was found between doneness levels of meat consumed and breast cancer risk; the adjusted ORs for very well-done versus rare/medium done were 1.54 (CI = 0.96–2.47 for hamburger, 2.21 (95% CI = 1.30–3.77) for beef steak, and 1.64 (95% CI = 0.92–2.93) for bacon. Women who consumed these three meats consistently very well-done had a 4.62-fold (95% CI = 1.36–15.70) greater risk than women who consumed them rare/medium-done. Breast cancer risk was also elevated with increasing intake of well- to very well-done meat. Consumption of well-done meats may play an important role in breast cancer risk.

In order to advance our knowledge on the role of HCA in cancer, it is critical to develop valid and accurate methods to assess both external and internal HCA exposure. Continuing efforts to improve questionnaires to evaluate external exposure to dietary HCAs, as well as to develop biomarkers of long-term internal exposure that can be measured in easily accessible tissues (e.g. protein or DNA adducts) is thus needed. To clarify complex relationships between genetic variants, HCA intake and risk of cancer will also require large studies. A new generation of large case-control and cohort studies using state-of-the-art methods to assess HCA exposure are currently underway and will provide important clues to these relationships in the near future. Better knowledge on metabolic pathways for HCAs and the functional importance of metabolic polymorphisms is also needed to identify combinations of genetic variants that could modify individual susceptibility to HCAs.

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